Justification
National Center for Research Resources

Authorizing Legislation - Sections 301, 479, 481 and 487 of the Public Health Service Act. Reauthorizing legislation will be submitted.

Budget Authority:

FY 1999 Actual		FY 2000 Estimate		FY 2001 Estimate		Increase or Decrease	
FTE	BA	FTE	BA	FTE	BA	FTE	BA
95	$464,759,000	106	$569,139,000	112	$602,728,000	+6	+$33,589,000

This document provides justification for the Fiscal Year 2001 non-AIDS activities of the National Center for Research Resources (NCRR). Justification of NIH-wide Fiscal Year 2001 AIDS activities can be found in the NIH section entitled "Office of AIDS Research (OAR)."

As the new millennium rolls in, doomsayers have predicted the end of the world as we know it--and in some respects they are right. Advances in computer technology, bioengineering, instrumentation design, and genetics will revolutionize biomedical research in the 21st century. Because of unprecedented progress in these areas, the compositions of several genomes of important research organisms already have been determined, and the human genome will be completely sequenced within a few years. Disease-causing genes may then be identified at a faster pace, and genetic or drug treatments designed.

NCRR ensures that biomedical scientists have access to the research tools and infrastructure that make these advances possible. As such, NCRR's mission is unique among the institutes and centers of NIH. While the other NIH institutes and centers focus on particular diseases, organ systems, or categories of research, NCRR alone has a trans-NIH mandate--to support the research infrastructure that enables all lines of biomedical inquiry.

NCRR programs are distributed across four areas--Clinical Research, Biomedical Technology, Comparative Medicine, and Research Infrastructure--reflecting the trans-NIH nature of NCRR activities. By providing scientists nationwide with access to advanced instrumentation, sophisticated research facilities, animal models, and biological materials, NCRR serves as a facilitator--or catalyst--for biomedical discovery.

One of NCRR's main objectives is to utilize scarce or expensive resources to the fullest by sharing them among several investigators, thereby increasing efficiency and saving manpower and research dollars. Each year more than 19,000 investigators, supported by more than $2.3 billion in competitive grant support from the other NIH components, use NCRR-supported research resources. To meet the needs of biomedical investigators for access to costly technologies, the NCRR collaborates with the

Department of Energy and the National Science Foundation (NSF) to provide access to high energy x-rays at the synchrotron facilities operated by those two agencies. In addition, NCRR provides access to advanced computing needs by partnering with the NSF-supported San Diego Supercomputer Center, one of the two National Partnerships for Advanced Computational Infrastructure currently supported by the NSF.

NCRR-funded resources have been critical to numerous projects that advance biomedical science. Many NCRR-supported discoveries have immediate benefits for patients; others help basic research move forward toward this ultimate goal. Recent NCRR-funded achievements are described below.

Science Advances

Bioengineering, Computers, and Advanced Instrumentation

Synchrotron Resources Enable Landmark Studies of Ribosome Structure

Proteins, the molecular workhorses of the cell, are assembled according to explicit molecular blueprints within a complex subcellular "factory" known as the ribosome. Scientists have long struggled to get a glimpse of these tiny factories, but high-resolution images have remained elusive, in part because ribosomal subunits proved too large and complex to be analyzed by x-ray crystallography. Now, thanks to the high-energy x-ray light that is available only from synchrotrons, two independent teams of investigators have probed crystals of ribosomal subunits and determined their three-dimensional structures in unprecedented detail. The structures offer significant insights into how ribosomes manage the assembly of protein chains and may also expedite discovery of effective antibiotics through structure-based drug design, since antibiotics often work by inhibiting ribosome function in bacterial cells.

Safely Imaging Live Embryos with Two-Photon Microscopy

Confocal microscopes, which use high-energy light (photons) to excite and image fluorescent probes in living cells, have revolutionized the study of cellular function. But because high-energy photons quickly damage living specimens and therefore restrict observation time, NCRR-supported scientists at Cornell University developed an alternative technique known as two-photon microscopy, which uses two low-energy, less-damaging infrared photons to simultaneously excite fluorescent probes. To evaluate the safety of the two-photon approach for long-term study of living specimens, researchers at the NCRR-supported Integrated Microscopy Resource at the University of Wisconsin, Madison, examined hamster embryos under the microscope, reimplanted the embryos into animals, and generated live offspring with no apparent developmental defects. Two-photon microscopy holds promise for tracking cell migration and gene expression during embryonic development and may even allow cancer researchers and clinicians to study tumor cell metastases through living tissue.

MRI of Cartilage May Aid Early Diagnosis and Treatment of Osteoarthritis

Osteoarthritis, a major cause of disability in the over-50 population, affects more than 20 million Americans, according to the American Arthritis Foundation, and imposes considerable morbidity on patients and expense on the health care system. There is no known cure for this debilitating disease, and current therapies focus only on symptomatic relief. To aid early diagnosis of osteoarthritis, and possibly allow effective evaluation of potential therapeutics, scientists at an NCRR-supported magnetic

resonance resource at the University of Pennsylvania are developing noninvasive techniques for detecting subtle degenerative changes in cartilage, which is the primary pathology associated with the disease. New sodium and proton magnetic resonance imaging (MRI) methods developed at the resource enabled detection and measurement of cartilage changes in an *in vitro* model of early manifestations of osteoarthritis. These promising new imaging methods may one day enhance physicians' abilities to diagnose and treat osteoarthritis before significant structural damage to the cartilage develops.

Rapid, Comprehensive Analysis of Protein Complexes

Some of the cell's most important activities occur within macromolecular complexes such as the ribosome, a protein-manufacturing "factory" that is itself made up of numerous unique proteins. To tease out and identify the many components of large protein complexes, scientists often isolate and then analyze each protein or gene individually--a process that can easily take years to complete. But now investigators assisted by the NCRR-supported yeast genome resource at the University of Washington, Seattle, have developed a rapid new process called DALPC (direct analysis of large protein complexes) that is capable of comprehensively identifying individual proteins in even the most complex macromolecular structures without first purifying each protein component. To validate the process, the investigators used DALPC to analyze a ribosome of the yeast *Saccharomyces cerevisiae* and identified more than 100 proteins in the large macromolecular structure, including a novel protein component of the yeast and human ribosomes. This new method will enhance understanding of complex biological phenomena and will greatly contribute to biological investigations in the post-genomic era.

New Insight in Muscle Elasticity

The modular protein titin, which is responsible for the passive elasticity of muscle, is subjected to stretching forces. Previous experiments on the elongation of single titin molecules have suggested that stretching force causes consecutive unfolding of each domain of the protein in an all-or-none fashion. But now researchers at an NCRR-supported resource have used a combination of genetically engineered protein models of titin and sophisticated simulation techniques to show that complete titin unfolding is preceded by a reversible, stretched intermediate state. Stretching of the titin model molecule by atomic force microscopy caused a 6 angstrom extension prior to complete unfolding. This partial unfolding can extend the molecule’s length by 15 percent before it unravels completely and is therefore likely to be an important component of titin elasticity. This new evidence provides insight into the role of titin in controlling muscle elasticity, especially in cardiac muscle. Titin and similar types of proteins are implicated in the etiology of several major diseases, such as heart insufficiency and cancer.

A Detailed Look at a Cellular Organelle

To completely appreciate and understand the function of an organism, it is necessary to visualize in as much detail as possible its cellular building blocks. The combination of electron microscopy and powerful computers has made it possible to look inside cells and obtain three-dimensional (3-D) images of their components. Using an electron microscope with a high-energy electron beam, NCRR-supported investigators at an electron microscopy center in Boulder, Colorado, prepared clear images of samples cut from cells that had been preserved for detailed structural study. After taking about 150 images of the same cellular region viewed from many directions, the researchers were able to assemble a 3-D reconstruction, or “tomogram,” of the Golgi complex, a system of sacs and vesicles, each

bounded by a membrane and stacked up to form an organelle. The Golgi complex is the organelle in which cells modify some of the proteins they have recently made, adding or removing sugars to make the proteins suitable for transport to a specific part of the cell or for export from the cell. Although the Golgi complex has been studied for more than 100 years, its precise 3-D structure had not been determined. With this type of imaging technology, scientists are able to build models that represent the membranes of the Golgi sacs and vesicles, showing how they are organized and connected. Ultimately the research may clarify cellular processes that are important for normal cell function and derailed in diseased cells.

Biology of Brain Disorders

Novel Brain Scanning Technique Detects Parkinson-like Condition

By studying a nonhuman primate model for Parkinson's disease, investigators at the New England Regional Primate Research Center have developed and evaluated an imaging tool that may allow early detection of brain cell loss that is characteristic of Parkinson's disease. The scientists employed imaging compounds that target dopamine-secreting neurons, which are progressively depleted in the brains of patients with Parkinson's disease. The new technique will allow scientists to mathematically model progressive degeneration of dopamine terminals, calculate the rate of degeneration, and predict when signs of the disease will likely appear, which would be a valuable diagnostic tool. Parkinson's disease affects an estimated 1 million people in the United States, according to the American Parkinson Disease Association; earlier detection may enable timely intervention to prevent, or perhaps even reverse, neuronal loss.

Nuclear Magnetic Resonance Reveals More Pieces of the Prion Puzzle

Prions are proteins normally found in the brain of all animals, but aberrant forms of the proteins are associated with deadly neurodegenerative diseases that leave the brain riddled with holes. Creutzfeldt-Jakob disease (CJD), a human prion disorder, can be acquired by eating tainted beef. Prion diseases of animals include scrapie in sheep and bovine spongiform encephalopathy (BSE, or "mad cow disease") in cows. The biological underpinnings of prion diseases are poorly understood, but a wealth of data suggests that pathologies arise when the three-dimensional structure of the normal cellular prion protein is converted--through an unknown mechanism--into an infectious pathogenic form. Using powerful new high-field nuclear magnetic resonance (NMR) spectrometers purchased via an NCRR Shared Instrumentation Grant, scientists identified the crucial parts of the prion protein that can change and cause the deadly diseases. Knowledge of these structural changes may also shed light on other neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, both of which are associated with abnormal aggregations of proteins and related molecules within neurons.

Gene Therapy Reverses Nerve Cell Atrophy

Decreases in cognitive function accompany the normal aging process in both humans and nonhuman primates. These regular aging features include impaired selective attention, executive functions, and some components of declarative memory. However, scientists have been unable to pinpoint changes in specific anatomical regions of the brain associated with these processes. Now NCRR-supported researchers at the University of California, San Diego; the Salk Institute for Biological Studies; and the California Regional Primate Research Center have shown that nerve cells in subcortical regions of aged rhesus monkeys degenerate, or atrophy. This cellular atrophy was almost completely reversible when

tissue grafts of genetically modified cells that produced human nerve growth factor were implanted in the brain of the aged monkeys. These findings have implications not only for cognitive function in normal aging, but also for preventing or restoring some of the cognitive decline in neurodegenerative conditions, such as Alzheimer's disease, in which this same system of cells is known to undergo profound atrophy and death.

Novel Approach Detects Changes in Brain Chemistry in Depressed Patients

Depression is among the most common mental disorders. According to the National Institute of Mental Health, depression affected more than 19 million adult Americans and cost society between $30 and $44 billion in 1990. Direct methods to study the neurobiology of brain structures involved with psychiatric disorders such as depression have not been available previously. Now NCRR-supported researchers at the University of Pittsburgh General Clinical Research Center have developed methods to assess changes in central nervous system metabolism in depression by examining brain function during sleep. Using a sophisticated imaging technique called positron emission tomography (PET), the investigators found that in healthy subjects the forebrain becomes relatively deactivated when a person falls asleep. In the transition from inactive (or non-REM) sleep to active (or REM) sleep, specific areas of the brain are reactivated. These are the same areas that are activated in normal motivational behavior and responsiveness to stress. Using this advanced PET technique, specific areas of the brain can be identified and studied in a variety of mood disorders as well as other diseases of the brain. The identification of these specific brain areas is a fundamental first step in future design of treatment.

Genetic Medicine

Genome-wide Search for Type 2 Diabetes Susceptibility Genes

Type 2 diabetes, which--according to the National Institute of Diabetes and Digestive and Kidney Diseases--affects 90 to 95 percent of the 16 million Americans with diabetes, is believed to result from a complex interplay between genetic and environmental factors. Scientists have recently identified several genes responsible for rare forms of type 2 diabetes but have not yet pinpointed major genetic contributors to the more typical condition. By studying DNA samples from 42 multigenerational families, each of which had at least two siblings who manifested type 2 diabetes before age 65, scientists have now identified a novel diabetes susceptibility locus on chromosome 1. More than 600 affected and unaffected family members were assessed for this study, which received critical support from the NCRR's General Clinical Research Center at the University of Utah. The investigators found that nonaffected siblings who harbor this newly identified susceptibility locus are 2.8 times more likely to develop diabetes than those who do not carry the gene(s). Identifying the genes that contribute to type 2 diabetes will help researchers to uncover the molecular basis of this disorder, which in turn may lead to improved treatment strategies and early identification of at-risk individuals.

Feasibility of Gene Therapy in Glaucoma

According to the Glaucoma Foundation, every year more than 50,000 Americans develop glaucoma, a disorder causing elevated pressure within the eye that can lead to blindness. Treatment for glaucoma involves lowering the intraocular pressure, which is the best documented neuroprotectant for the optic nerve and retinal ganglion cells in this disease. But the eye is an attractive target for gene therapy because of its well-defined anatomy, immunoprivilege, and accessibility. In the eye, with its laminar structure, a specific gene may be introduced into a sufficient number of cells to have a therapeutic

effect. Investigating this therapeutic approach, NCRR-supported researchers at the University of Wisconsin, Madison Medical Center, have demonstrated the feasibility of gene transfer to the eyes of two different animal models. A herpes viral vector was used as a vehicle to deliver the *lacZ* reporter gene to the eyes of living anesthetized cats and rats. The scientists were able to visualize the gene incorporation by a color reaction caused by the enzyme coded by the *lacZ* reporter gene in the viral vector. This gene therapy approach may be used to achieve expression of other foreign genes within the tissues of the eye that may influence the intraocular pressure and retinal cell biology, thereby preventing, delaying, or minimizing glaucomatous damage to the optic nerve.

Unraveling the Causes of Excessive Insulin Production in Newborns

Neonatal hyperinsulinism (HI) is a disorder of pancreatic function characterized by failure to suppress insulin secretion when the blood sugar level is low. The disorder can result in brain damage or death if inadequately treated. One cause of HI is an abnormal increase in the number of insulin-producing cells, either uniformly distributed throughout the pancreas (diffuse HI) or present in distinct regions, or foci (focal HI). The two forms were until now only discernable by surgery in which a portion of the pancreas is removed. Using gene mapping techniques, NCRR-supported investigators at Children's Hospital of Philadelphia General Clinical Research Center have now shown that focal HI is caused by a combination of a loss of a section of the maternal chromosome 11p and a mutation in a paternally derived gene known as *SUR-1*. With medical treatment, patients having both the paternal mutation and the maternal chromosome loss enter clinical remission in about 16 months; patients who have retained *SUR-1* genes from both parents require about 4 years for remission. The remission is apparently caused by self-destruction of the abnormal insulin-producing cells. The capacity to use non-surgical approaches to diagnose this life-threatening disease and predict its responsiveness to a therapeutic agent will improve the care of infants afflicted with neonatal hyperinsulinism.

New Approaches to Pathogenesis

Transplants Save Lives of Children with Severe Immune Disorder

Children born with severe combined immunodeficiency (SCID), a rare syndrome marked by a profound lack of immune system cells, often die from common infections within the first year of life. A new study now reports that most babies with SCID can survive, sometimes even into their teenage years, if given a bone marrow transplant from a family member within the first 3.5 months of life. By analyzing the outcomes of 89 SCID patients who had received bone marrow transplants at Duke University, scientists at the NCRR-supported General Clinical Research Center discovered that parents as well as siblings of SCID-affected babies can be successful marrow donors, as long as mature T cells are removed from the graft before transplantation. Removing mature immune cells keeps them from attacking the patient's vital organs--a serious complication of transplantation known as graft-versus-host disease. Newborn screening for SCID may allow affected babies to receive a life-saving transplant within the first few days of life.

New Avenues for Development of Therapeutics

Forcing Cancer Cells to Commit Suicide

Basic research into the cell cycle--the series of precise, predetermined steps that underlie cell division--has revealed an Achilles heel that is unique to cancer cells. Studies have shown that most tumor cells lack a protein "brake" that temporarily halts the cell cycle in normal cells. Without this brake, cancer cells proliferate unchecked, and abnormal levels of regulatory proteins arise. Now scientists have discovered a way to exploit these abnormalities and cause cancer cells to work against themselves. The researchers synthesized a small peptide that crosses the cell membrane, inhibits specific regulatory proteins, and instructs cancer cells--but not normal cells--to self-destruct. Cell-cycle analyses in these studies were made possible with a multi-laser, high-speed cell sorter purchased via an NCRR Shared Instrumentation Grant. Since the protein "brake" is deactivated in virtually all cancer cells, a therapy targeting this pathway may have wide application to a variety of tumors.

Biomolecular Underpinnings of Acute Human Leukemias

Acute human leukemias are associated with chromosomal alterations of genes that encode two related proteins, the alpha and beta forms of core binding factor (CBF). During its normal function, CBFalpha binds to DNA and helps regulate genes that aid blood and bone development, whereas CBFbeta complexes with CBFalpha to increase affinity for the DNA binding sites. Using advanced nuclear magnetic resonance instruments purchased with an NCRR Shared Instrumentation Grant, scientists analyzed the structures of CBFalpha and CBFbeta proteins and identified the specific portion of CBFalpha that interacts with DNA. In connection with development of leukemia, the DNA-binding portion of the CBFalpha gene becomes associated with other genes called transcription factors. These studies of normal and mutated CBFs offer new insights into leukemogenesis and may enable development of highly specific, and hence less toxic, forms of leukemia therapy.

Health Disparities

Association of Gene Polymorphisms, Tobacco Carcinogens, and Lung Cancer

Cigarette smoking is the major risk factor for lung cancer, but susceptibility also depends on the genetic makeup of each individual. Before the carcinogens in tobacco smoke can wreak havoc on the body, they must be activated by specific enzymes, and inherited variations in these enzymes can alter an individual's risk for cancer. By studying 341 patients with lung cancer and 456 healthy individuals, researchers at the University of Hawaii, supported in part by NCRR's Research Centers in Minority Institutions program, determined that smokers who carried a particular variant of a carcinogen-activating enzyme had a 2.4-fold increased risk of squamous cell carcinoma (SCC), a common type of lung cancer. The risk of SCC escalated even further (3.1-fold) if the smoker also lacked both copies of a carcinogen-deactivating gene known as GSTM1. In contrast, variants of a different enzyme were associated with a reduced overall risk of lung cancer and, in particular, a reduced risk of adenocarcinoma, which is the most common form of lung cancer today. The findings help to clarify the roles of specific tobacco smoke constituents and metabolic pathways in the causation of common types of lung cancer.

Future Research Directions

Bioengineering, Computers and Advanced Instrumentation

Imaging

Many human diseases are known to arise from a combination of environmental, genetic, and other factors that interact in a complex, variable manner within an individual. Understanding these complexities requires a unified, integrated approach that examines the appearance and progression of a specific pathology. Improved imaging systems are needed to thoroughly investigate the pathophysiology of human disease by using small animals and nonhuman primate models of human disease. Microscopic imaging with computed tomography, magnetic resonance imaging (MRI), positron emission tomography, and nuclear imaging present unique challenges to the engineer, physicist, and biologist. NCRR proposes to support further technology development to make these imaging tools more affordable and user friendly along with higher throughput capabilities and greater resolution.

Recent advances in functional magnetic resonance imaging (fMRI) methodologies, coupled with increased recognition of the close ties between vascular physiology and brain function, have led to a virtual revolution in neuroscience research. The potential for studying the integrated behavior of the normal and diseased brain has never been greater. Unfortunately, access to academic health center MRIs is extremely limited or nonexistent since that equipment is dedicated to patient service 24 hours a day throughout the entire week. Existing NCRR-funded biomedical technology resource centers for MRI can serve some of the research community, provided the centers can be equipped with advanced fMRI instruments dedicated to this purpose. Additional regional MRI resources are needed to assure that the promising preliminary research on neurodegenerative diseases with stem cells and other therapeutic approaches can be attained. The NCRR resource centers have the technological know-how and intellectual expertise to assist investigators in their effort to arrest, reverse or cure neurodegenerative diseases, including Parkinson's and Alzheimer's Disease.

Bioinformatics

NCRR proposes to establish biomedical informatics centers that respond to recommendations of the June 1999 Working Group report to the NIH Director on Biomedical Computing (The Biomedical Information Science and Technology Initiative, BISTI). The Centers will advance research in particular areas of biomedicine, focusing on those in which computation is becoming increasingly essential. At least one of these centers will also provide access to high-end (teraflop) computational capability dedicated to biomedical research and essential for modeling and simulation of significant biological processes at the molecular, cellular, and tissue levels of organization.

One important goal of this program will be to develop and integrate the use of computational tools to meet the important challenges of biomedical research. Concurrently, the Centers will create homes for interdisciplinary teams, and those teams will establish nurturing environments for exploration and education. Establishing these Centers will send a powerful message, both in academe and within the NIH community itself, about the necessity and importance of computation and the value of interdisciplinary research for addressing complex biomedical research problems.

To make the growing body of biological data available in a form suitable for study and use, NCRR proposes to establish a program directed toward the principles and practice of information storage,

curation, analysis, and retrieval in accord with Recommendation #2 of the June 1999 Working Group report on Biomedical Computing (The Biomedical Information Science and Technology Initiative, BISTI).

The information that biomedical researchers--both basic and clinical--are amassing in profuse quantities today creates enormous digital repositories of information. The scale of these databases swamps all the information collected heretofore. The infrastructure needed to make them available is phenomenal: a single biomedical laboratory can produce up to 100 terabytes of information a year--about the same as the information in one million encyclopedias. In order to be useful, the data must be indexed and stored, and the challenges for data analysis and abstraction are formidable.

The vast databases now being generated by several genome projects and studies to discern gene function will continue to provide vast sets of raw data for addressing considerably more complex biomedical questions in the near future. Through manipulation of various genomic databases networked to other biologically relevant data, much of the biology done in the next century will require complex analyses and visualization tools. This trend will include determination of protein structure and function as well as analyses of cell and organism physiology made accessible by new microarray and other technologies. As the amount of data grows, the tools to compare and manipulate the data become more important. These tools include the design and establishment of new database structures, uniform access to heterogeneous databases, data mining, analysis, and visualization in three and four dimensions.

Although development of bioinformatics tools will be an ongoing activity for the foreseeable future, a number of areas appear ripe for exploitation, including tools for sequence and macromolecular analysis, deducing regulatory structures, predicting molecular structure and function, and annotating molecular functions. Such approaches promise to aid development of advanced data-mining tools to explore large databases, with a goal of discovering new relationships but usually with no specific target defined at the outset.

In addition to specific bioinformatics tools, there is a critical need for multidisciplinary training in this field. Investigators already trained in the biological or biomedical sciences will need to enhance their knowledge with additional training in bioinformatics or computational biology. Non-biologists, such as those with degrees in informational or computer sciences, may wish to supplement their education with training in the life sciences. This effort impacts both basic and clinical research investigators and their rapidly growing need for new bioinformatics tools, including databases capable of handling heterogenous data and very large data objects.

Synchrotron Resources

Synchrotron resources are having an enormous impact on structural biology and drug design. Compared to laboratory-based x-ray sources, synchrotrons provide increased rates of data acquisition, higher overall data quality, and the capacity to work with small crystals. Furthermore, enormously powerful techniques such as multiwavelength anomalous diffraction (MAD) and three-beam phase studies require the capacity to adjust the wavelength of x-rays as can be done at synchrotron sources. More than half of all biomolecular structures reported in recent years have been solved with support from synchrotron facilities. The number of NIH users at NCRR-supported synchrotron radiation

resources doubled between 1995 and 1997, and requests for access to these facilities are increasing at an exponential rate. Past and projected growth rates of synchrotron facility use for structural biology indicate that a substantial shortfall in beamtime available to the NIH user community will occur during the next 4-5 years. This two-pronged initiative is put forth by NCRR to meet the anticipated needs of the biomedical research community.

Third-generation synchrotrons use undulators to produce high brilliance x-ray beams that are required for data collection on small, weakly diffracting crystals such as those characteristic of large macromolecular assemblies and of membrane proteins. NCRR proposes the development and commissioning of a new MAD-capable beamlines at the Advanced Photon Source (APS) that will focus on novel applications of undulator radiation in structural biology. This will combine new developments in beamline design, x-ray detectors, cryocrystallography, robotics, and computational software to address the next generation of technically challenging structural biology projects. Additionally, four high throughput MAD-capable beamlines will be developed at the Advanced Light Source (ALS) based on installation of new superconducting bending magnets. These beamlines will be particularly useful for general crystallography and work involving microcrystals, thereby addressing the problem of oversubscription.

Staffing levels at even the most advanced facilities cannot support more than 16 hours per day of operation for experienced users, and many inexperienced users are now applying for space and require expert assistance. In addition to extra personnel and special resources, such as robotics, the rapid production of crystallographic structures from diffraction data depends on new approaches to solving crystallographic phasing problems.

Bioengineering

By applying engineering principles and techniques to biological problems, bioengineering can enhance understanding of complex living systems and elucidate fundamental principles in biology. NCRR proposes to advance bioengineering by funding the development of innovative biomedical applications of nanotechnology, which involves manufacture of tools, instruments, and machines in the nanometer-size range (1 nanometer is one-billionth of 1 meter). Research equipment of this size has important applications in biosensors and drug design and delivery. NCRR also proposes to fund development of new imaging technology that will integrate organ and tissue structure and development with underlying molecular-level properties. This initiative will also include support for development of new methods and algorithms to perform mathematical modeling of complex biological systems. Mathematical simulations provide a rational approach for integrating knowledge gained from molecular-level to whole organism biological studies. Such integrations will play important roles in diagnosing and developing treatments for complex diseases.

Genetic Medicine

Gene function may be explored through phenotypic assessment of genetically altered animals which serve as models of human disease. NCRR proposes to support the development, coordination, and expansion of technologies and resources for phenotyping and analyzing gene expression in genetically modified and normal animal models commonly used in biomedical research. Collections of cDNAs that represent all or a substantial proportion of genes of research interest for microarray studies of gene function for several animal models will be provided through national resources. This initiative will

provide critical infrastructure to significantly enhance our understanding of disease mechanisms and potential approaches to prevent, alleviate or treat disease. In addition, the state-of-the-art research facilities and expert investigators will provide a venue for young veterinarians to work closely with mentors to enhance their research capabilities in comparative pathology and laboratory animal medicine.

Research using model organisms to gain knowledge of human gene function is progressing at a phenomenal rate. Complete sequencing of the genomes of the yeast *S. cerevisiae* and the roundworm *C. elegans*, and the near-complete genome sequencing of several additional organisms, provides vast stores of data for investigators who study these organisms as models of human development and disease. The rapidly expanding data on genes and gene function must be readily accessible for investigators to compare genomic data across model organisms. Databases exist for many model organisms, including yeast, *C. elegans, Xenopus, Drosophila,* and zebrafish. However, curation, standardized data entry and scientific editing need to be enhanced to maintain the utility of these databases. NCRR proposes to increase research support for this rapidly expanding need for database support for the zebrafish, *Xenopus*, *Saccharomyces*, *Drosophila*, and *C. elegans*. In addition, data must be linked across databases and include links to histological atlases as well as to databases for gene expression, including metabolic and signaling pathways. NCRR proposes to provide support for additional resource staff to maintain and validate several expanded model organism collections along with adding staff to complementary databases for curation, validation and data mining.

Biological tools known as vectors are used in gene therapy to carry therapeutic genetic materials into cells that have genetic defects. To improve the selection of vectors appropriate for different types of cells, investigators are continually working to develop new types of gene vectors. NCRR proposes to support efforts to optimize and standardize production methods as well as to support the production of a single vector reference standard. Separately, NCRR proposes to support development of a generally accessible drug master file that will contain pharmacology/toxicology and biodistribution data for recombinant lentiviral vectors. Availability of standardized materials and test data will save time and expenses and ultimately result in safer, more efficient treatment of patients.

Health Disparities

In accordance with the new national race and health disparities initiative, six focus areas have been selected in which racial and ethnic minorities experience serious disparities in access to health care, which has contributed to the disproportionately higher rates for infant mortality, and less-than-optimal cancer screening and management, along with higher rates or more advanced cases of cardiovascular disease and stroke, diabetes, HIV infection, and lower rates of adult and child immunization. NCRR proposes to help alleviate health disparities by competitively establishing several Comprehensive Centers on Health Disparities (CCHD) at medical schools located at universities that have an NCRR-supported Research Centers in Minority Institutions (RCMI) facility. The NCRR CCHD initiative will focus on all six areas but with increased emphasis on cancer screening and management and cardiovascular disease and stroke. Investigators at the CCHDs may assess disease incidence, conduct risk factor assessment, and aid disease prevention efforts in the indigenous populations associated with the respective institutions. Studies may also focus on cultural, environmental, and other factors that contribute to the disparity in research and treatment for diseases that are present disproportionately in minorities. This effort will be in partnership with appropriate categoric NIH institutes and with nearby

NCRR General Clinical Research Centers.

Research Capacity

With today's increased emphasis on the use of animals, particularly rodents, in biomedical research, there is a concurrent need for centralized, shared resources to support specialized technologies and efforts that are beyond the scope of an individual research project, or even several projects. NCRR proposes to establish regional networks of comparative medicine and integrative biology core grants to provide NIH-funded investigators with additional, shared support to enhance their own and their institution's capability to conduct animal-based research. These core grants will be divided into discrete units or modules, each devoted to a specific activity that would be impractical or less efficient to support on an individual research project grant. Modules are based on functions, such as laboratory services, shared equipment, and technical support. The cores will also serve as bases for research training in comparative pathology and laboratory animal medicine.

Sophisticated research facilities are required to perform biomedical investigations critical to the health of the American people, but new construction or renovation of current facilities is urgently needed. According to a 1998 National Science Foundation survey, biomedical research-performing institutions had to defer $5.6 billion worth of needed construction because of insufficient funds. NCRR will continue to provide funds on a competitive basis to upgrade biomedical research facilities to improve the nation's infrastructure in this critical area. The funds will be provided competitively through the NIH extramural construction grant program, which is a matching NIH-wide program administered by NCRR.

NCRR has administered an NIH-wide matching Animal Facility Improvement program for several years. A major part of current facilities-related renovation activity reflects the fact that genetically altered rodents are increasingly used to enhance our understanding of gene function. Modern facilities are required to perform genetic research and specialized studies with rodents, nonhuman primates and other animal models. NCRR proposes to upgrade space for sophisticated research for functional genomics, including phenotyping. To assist research-performing Historically Black Colleges and Universities and other minority-serving institutions in bringing their animal research facilities up to AAALAC standards, NCRR proposes a special initiative to address this problem. Currently, only one of those institutions' animal research facilities is AAALAC accredited.

Career Development

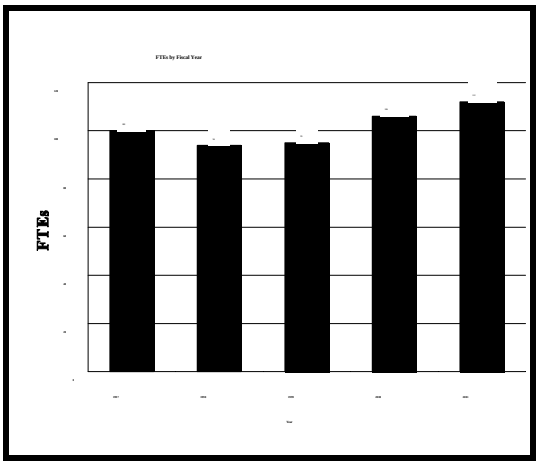
Studying patients in clinical research projects is essential for finding causes and cures for diseases and for evaluating new drugs, but fewer young physicians appear to be pursuing research careers. NCRR has initiated several programs to address the need for more clinical investigators and proposes to extend those in FY 2001. NCRR proposes to expand support for a year-long medical student mentored clinical research training program, usually between the third and fourth years of medical school. The intent of this program is to serve as a catalyst for young physicians to pursue careers in patient-oriented research. This program will foster and possibly reinforce an interest in clinical research among promising medical students. The institutional GCRC or the RCMI-funded Clinical Research Center will serve as a focal point for patient-oriented research, through mentored didactic training and "hands-on" research. This new program, ***Medical Student Clinical Research Program***, will support up to 90 students per year. The ***Clinical Research Scholars Program*** for young physicians

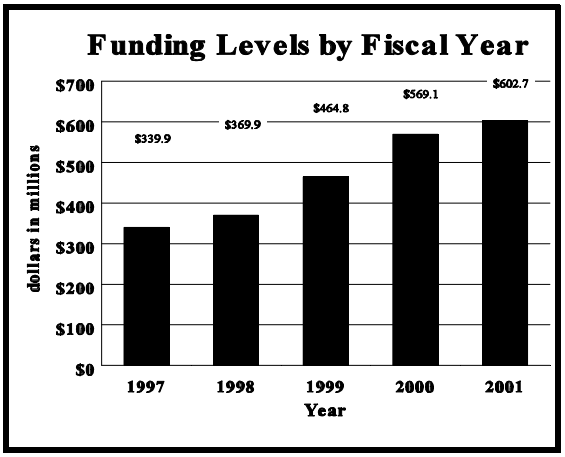
and dentists provides a one-year or two-year program immediately after clinical training that could lead to a M.S. or M.P.H. degree to attract interested but uncommitted investigators. NCRR will continue to participate in the ***Mentored Patient-Oriented Research Career Development Awards Program,*** the grant mechanism which now provides support to physicians and dentists for up to five years at GCRC sites. This very successful program was formerly known as the Clinical Associate Physician (CAP) program.

Recent and rapidly growing advances in molecular biology have led to production of genetically modified animals of several species. Assessment of the visible, or phenotypic, manifestations of genetic changes rely on individuals trained in whole-animal biology and the variety of specialized techniques required for these types of studies. Veterinarians are ideally suited, through their professional training, to evaluate such issues, but many require further instruction in the specialized techniques, methodologies, and research approaches used in phenotyping efforts. NCRR proposes to address this issue with two strategies: Initiate a one-year program for veterinary students that will provide a mentored biomedical research experience at research-intensive institutions; and expand the Special Emphasis Research Career Award program to support several more veterinarians after their professional and clinical training. These approaches are intended to address the inadequate number of research-trained veterinarians who participate in biomedical research. This constitutes a critical shortage since animal-based research is included in nearly half of all NIH investigator-initiated awards.

Budget Policy

The Fiscal Year 2001 budget request for the NCRR is $602,728,000, excluding AIDS, an increase of $33,589,000 and 5.9 percent above the FY 2000 level. Included in this total is $23,950,000 for the following NIH Areas of Special Emphasis: Genetic Medicine ($3,000,000), Bioengineering, Computers, and Advanced Instrumentation ($14,000,000), Health Disparities ($1,000,000), Research Capacity ($750,000), and Career Development ($5,200,000).
A five year history of FTEs and funding levels for NCRR are shown in the graphs below:





One of NIH's highest priorities is the funding of medical research through research project grants (RPGs). Support for RPGs allows NIH to sustain the scientific momentum of investigator-initiated research while providing new research opportunities. To control the growth of ongoing commitments and support planned new and expanded initiatives, the Fiscal Year 2001 request provides average cost increases of 2 percent over Fiscal Year 2000 for competing RPGs. Noncompeting RPGs will receive increases of 2 percent on average for recurring costs, a reduction of 1 percent below committed levels of support. This strategy will ensure that NIH can maintain a healthy number of new awards, especially for first time researchers. The Fiscal Year 2001 request for NCRR includes funding for 49 competing RPGs, 92 noncompeting RPGs, and 74 Small Business Innovative Research awards.

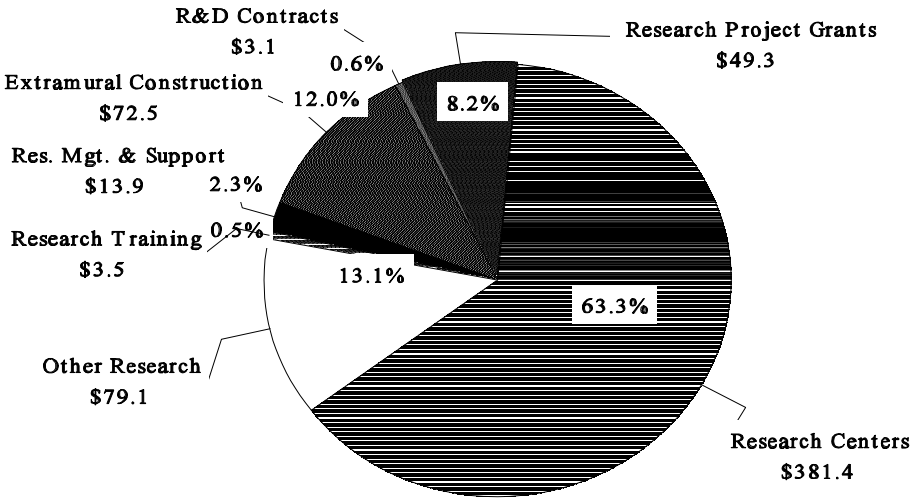
Promises for advancement in medical research are dependent on a continuing supply of new investigators with new ideas. In the Fiscal Year 2001 request, NCRR will support 87 pre- and postdoctoral trainees in full-time training positions. Stipends will increase by 2.2 percent over Fiscal Year 2000 levels.

The Fiscal Year 2000 request includes funding for 222 research centers, 321 other research grants, including 10 new clinical career awards, and 11 R&D contracts.

The mechanism distribution by dollars and percent change are displayed below:

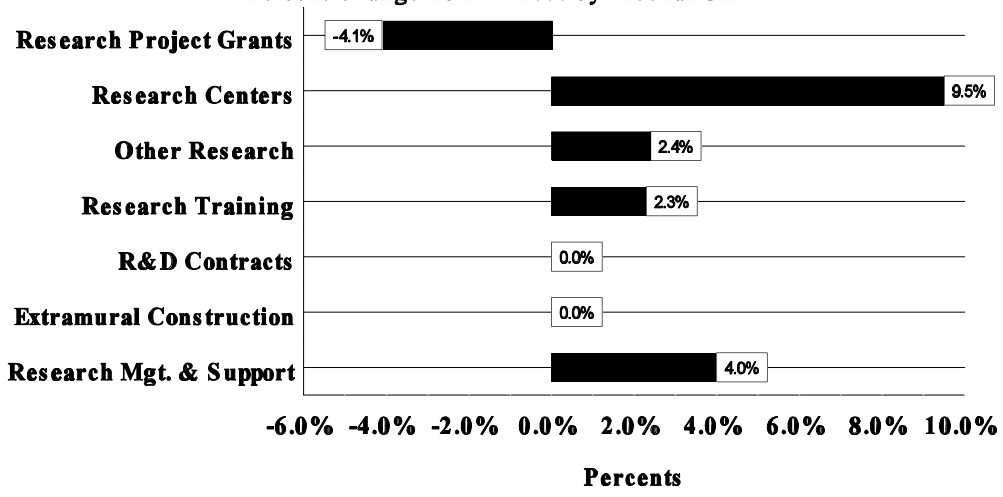
FY 2001 Budget Mechanism

(Dollars in Millions)



FY 2001 Estimate

Percent Change from FY 2000 by Mechanism



Story of Discovery: Synchrotrons Illuminate Atomic Architecture of Life

The principle "form follows function" is espoused by designers and architects who believe that the shapes of objects should suit their intended use. This principle also holds true in biology. In fact, form is so critical to the function of biological molecules that even the slightest alteration in a protein's three-dimensional (3-D) structure can produce life-threatening disorders such as sickle cell anemia, Lou Gehrig's disease, or an abnormal heartbeat. By studying the minute details of molecular shape and its impact on the human body, structural biologists have gained a deeper understanding of the molecular bases of disease and have used this knowledge to devise improved therapies for diseases ranging from AIDS to the common cold. Many of these discoveries depended on the use of a very bright and versatile type of light known as synchrotron radiation.

Synchrotron radiation is produced when electrons or other subatomic particles are forced to accelerate inside a huge circular chamber, usually ranging from 200 feet to about 1 mile in circumference. As the particles accelerate around the path, they radiate a wide spectrum of light, including intense and brilliant x-rays. When physicists first observed synchrotron radiation in the late 1940s, they considered it more of a nuisance than an asset; the massive synchrotron facilities were originally built for studies of subatomic particles, and the radiation was regarded as an unwanted energy leak. But by the early 1970s, a handful of researchers had recognized that this intense type of light could be harnessed to probe the structures of matter--including biological molecules--by x-ray techniques, and they rallied to build laboratories adjacent to the synchrotron rings to tap this otherwise-wasted resource.

X-ray diffraction had been used since the 1950s to uncover the structures of dozens of biologically important compounds at near-atomic resolution, including hemoglobin, myoglobin, vitamin B12, and DNA. By shining x-rays through crystallized molecules from many different angles and then capturing the patterns produced by diffracted x-rays, biologists were able to calculate how the x-rays had been deflected, and from this mathematically deduce the 3-D, atomic structures of the crystallized molecules. However, each structure took several years--or even decades--to solve.

Biologists quickly discovered that synchrotron radiation offered many advantages over x-rays produced by conventional laboratory devices. Because synchrotron radiation is at least 1,000 times brighter, vast quantities of data could be collected more rapidly from smaller crystals. And because synchrotron radiation is also tunable, researchers could select specific wavelengths for their studies. Despite improvements, x-ray crystallography remained a tedious and frustrating procedure, in part because available instruments and techniques lacked the desired sensitivity.

NIH provided a critical boost to the emerging field in 1980 when it funded one of the nation's first synchrotron resources dedicated solely to biomedical research at the Stanford Synchrotron Radiation Biotechnology Resource. This facility pioneered a new approach to determine directly the phases in protein x-ray diffraction patterns (now usually called MAD phasing). When later combined with techniques of genetic engineering, a powerful new tool for structural biology was created.

Physicists and materials scientists are still the largest user group at U.S. synchrotrons, but the number of structural biologist users has grown dramatically over the past decade, approaching about one-third of the total utilization in 1998. The core operation of four national facilities is funded by the U.S. Department of Energy, and another site at Cornell is funded by the National Science Foundation. In addition to the Stanford facility, NIH now supports beamlines at the following synchrotron radiation facilities: the BioCARS Synchrotron Structural Biology Resource and the Biophysics Collaborative Access Team (BioCAT) in Chicago; the Regional Center for Time-Resolved Synchrotron Spectroscopy and the Macromolecular Crystallography Resource of the National Synchrotron Light Source, both located at Brookhaven National Laboratory in New York; and the Macromolecular Diffraction Biotechnology Resource (MacCHESS) at Cornell University. This arrangement reflects the interdependence of complex research infrastructures across Federal agencies.

Resource (MacCHESS) at Cornell University. This arrangement reflects the interdependence of complex research infrastructures across Federal agencies.

Technological advances made at NIH-supported synchrotron resources--combined with enormously improved computing power and molecular biology technologies such as those involving recombinant DNA --have transformed x-ray crystallography from a laborious undertaking to a powerful research tool for molecular biology. In 1970, fewer than a dozen protein structures had been solved at atomic resolution; today that number surpasses 8,000, with solvable structures--such as the ribosome--becoming increasingly large and complex. More than half of the almost 1,600 new molecular structures solved in 1998 utilized synchrotron radiation.

One critical protein structure solved a decade ago led directly to discovery of the highly effective and widely used anti-AIDS drugs known as protease inhibitors. Scientists working in part at the NIH-supported MacCHESS synchrotron resource at Cornell University in New York determined the structure of the HIV protease at atomic resolution with candidate drugs that could fit snugly into the protease's active site and block its activities. Together with numerous similar studies at other synchrotrons and in home laboratories, this work led to development of the next generation of protease inhibitors, several of which are now in clinical use.

Genome scientists are shifting their focus from genetic structure to function, and structural biologists are increasingly pursuing the functional implications of molecular structure. Such studies received a significant boost in 1996 when the Advanced Photon Source (APS) near Chicago opened, a third-generation synchrotron that produces hard x-rays that are two orders of magnitude brighter than those generated by older, second-generation synchrotrons.

Because synchrotron light is pulsed, it is ideal for time-resolved studies that produce brief "movies" of functioning molecules, capturing freeze frames of molecular motions that normally occur too rapidly to be seen. While older synchrotron sources also produce short pulses, they are not sufficiently bright to capture fast protein activities in real time. The new, third-generation synchrotrons like APS deliver extremely bright pulses that last for only 100 picoseconds, or trillionths of a second, each containing sufficient numbers of photons to record a "frame" of a movie. This allowed NIH-supported scientists to produce the world's first 3-D x-ray movie of a molecular reaction on a timescale of nanoseconds, or billionths of a second. The movie revealed how the molecule myoglobin changes its shape as it performs its primary function--capturing and releasing oxygen in muscle cells.

As the brilliant x-rays continue to find new and unanticipated applications, demand for access to biomedical synchrotron resources will continue its exponential climb to unravel the molecular basis of disease and to facilitate novel drug development.